

FILE 'STNGUIDE' ENTERED AT 11:26:17 ON 02 FEB 2005

FILE 'MEDLINE, BIOSIS, CAPLUS, AGRICOLA, ESBIODBASE, AQUASCI' ENTERED AT 11:27:31 ON 02 FEB 2005

D 100, 107 110, 97 BIB AB

FILE 'STNGUIDE' ENTERED AT 11:27:32 ON 02 FEB 2005

FILE 'MEDLINE, BIOSIS, CAPLUS, AGRICOLA, ESBIODBASE, AQUASCI' ENTERED AT 11:32:39 ON 02 FEB 2005

D 65 103 2 29 40 54 BIB AB

FILE 'STNGUIDE' ENTERED AT 11:32:41 ON 02 FEB 2005

INDEX '1MOBILITY, 2MOBILITY, ABI-INFORM, ADISCTI, AEROSPACE, AGRICOLA, ALUMINIUM, ANABSTR, ANTE, APOLLIT, AQUALINE, AQUASCI, AQUIRE, BABS, BIBLIODATA, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, BLLDB, CABA, CANCERLIT, ...' ENTERED AT 11:41:47 ON 02 FEB 2005

SEA (INTRAOPERATIVE OR OPERAT? OR SURGER? OR BIOPS?) AND (PCR O

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| 7 | FILE TULSA |
| 11 | FILE UFORDAT |
| 20 | FILE ULIDAT |
| 46151 | FILE USPATFULL |
| 3119 | FILE USPAT2 |

ACCESSION NUMBER: 2004:33869 BIOSIS
 DOCUMENT NUMBER: PREV200400031975
 TITLE: **HELICOBACTER PYLORI-MEDIATED REGULATION OF INTERLEUKIN-8 (IL-8) TRANSCRIPTION IN GASTRIC EPITHELIAL CELLS: ROLE OF APURINIC/APYRIMIDINIC ENDONUCLEASE-1/REDOX FACTOR-1 (APE-1/REF-1).**
 AUTHOR(S): O'Hara, Ann [Reprint Author]; Ding, Song-Ze; Ryan, Kieran; Mitra, Sankar; Izumi, Tadahito; Mifflin, Randy; Crowe, Sheila E.
 CORPORATE SOURCE: Charlottesville, VA, USA
 SOURCE: Digestive Disease Week Abstracts and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 353. e-file.
 Meeting Info.: Digestive Disease 2003. FL, Orlando, USA. May 17-22, 2003. American Association for the Study of Liver Diseases; American Gastroenterological Association; American Society for Gastrointestinal Endoscopy; Society for Surgery of the Alimentary Tract.
 DOCUMENT TYPE: Conference; (Meeting)
 Conference; (Meeting Poster)
 Conference; Abstract; (Meeting Abstract)
 LANGUAGE: English
 ENTRY DATE: Entered STN: 7 Jan 2004
 Last Updated on STN: 7 Jan 2004
 AB Background: We have previously shown that *Helicobacter pylori* infection increases expression of IL-8 and apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE-1/Ref-1) in human gastric epithelial cells. APE-1/Ref-1 repairs oxidatively damaged DNA and reductively activates transcription factors including the activator protein (AP)-1 complex (c-Jun/c-Fos) and NF-kappaB. The aim of this study was to examine whether APE-1/Ref-1 modulates IL-8 gene expression in *H. pylori*-infected gastric epithelial cells. Methods: *H. pylori* (Hp) 26695 or the *cag* PAI deficient isogenic mutant, 8-1, for varying times. IL-8 was measured in cell supernatants by ELISA and EMSAs were used to examine AP-1 and NF-kappaB binding in nuclear extracts. APE-1/Ref-1 expression was assayed by western blot and by real-time RT-PCR (**Cepheid Smart Cyclor**). The effect of APE-1/Ref-1 overexpression was assessed in AGS cells transfected with an APE-1/Ref-1 expression vector and co-transfected with a luciferase-linked IL-8 reporter construct containing AP-1 and NF-kappaB binding sites (-1498/+44 IL-8 luc). Results: Both strains of *H. pylori* increased APE-1/Ref-1 protein and mRNA expression but only the *cag* PAI bearing strain induced IL-8 secretion. Compared to other stimuli, Hp 26695 had the greatest and most rapid effect on AP-1 and NF-kappaB binding. Enhanced IL-8 transcription was detected in cells that overexpressed APE-1/Ref-1 compared to cells with basal levels of APE-1/Ref-1. Hp 26695 induced higher levels of IL-8 transcription compared to uninfected controls and this difference was further increased in cells overexpressing APE-1/Ref-1. Overexpression of APE-1/Ref-1 was confirmed by western blot and real-time PCR. Conclusion: These results indicate that APE-1/Ref-1, which is increased in response to *H. pylori* infection and oxidative stress, plays a role in the transactivation of IL-8 transcription. Our findings suggest that *H. pylori* induced transcription factor binding activity is mediated by APE-1/Ref-1 and together, these data further implicate APE-1/Ref-1 as playing a pivotal role in regulating the epithelial response to *H. pylori* infection. Supported by RO1 DK61769-01..

L1 ANSWER 7 OF 16 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2003:545152 BIOSIS
 DOCUMENT NUMBER: PREV200300546468
 TITLE: Detection and quantification of total and potentially virulent *Vibrio parahaemolyticus* using a 4-channel multiplex real-time PCR targeting the *tl*, *tdh*, and *trh* genes and a novel PCR internal control.

AUTHOR(S) : Vickery, M. C. L. [Reprint Author]; Blackstone, G. M.
[Reprint Author]; Nordstrom, J. L. [Reprint Author];
Depaola, A. [Reprint Author]
CORPORATE SOURCE: Gulf Coast Seafood Laboratory, U.S. FDA, Dauphin Island,
AL, USA
SOURCE: Abstracts of the General Meeting of the American Society
for Microbiology, (2003) Vol. 103, pp. Q-082.
<http://www.asmtusa.org/mtgsrsrc/generalmeeting.htm>. cd-rom.
Meeting Info.: 103rd American Society for Microbiology
General Meeting. Washington, DC, USA. May 18-22, 2003.
American Society for Microbiology.
ISSN: 1060-2011 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Nov 2003
Last Updated on STN: 19 Nov 2003
AB *Vibrio parahaemolyticus* (Vp) is an estuarine bacterium that is the leading
cause of shellfish-associated cases of bacterial gastroenteritis in the
U.S.. The thermolabile hemolysin gene (tl) is a species-specific marker
for *V. p.*; the thermostable direct hemolysin gene (tdh) and t

Using molecular beacons to detect single-nucleotide polymorphisms with real-time PCR.

AUTHOR: Mhlanga M.M.; Malmberg L.

CORPORATE SOURCE: L. Malmberg, Department of Medical Sciences, Molecular Medicine, Uppsala University Hospital, S-75185-Uppsala, Sweden. loma0102@student.uu.se

SOURCE: Methods, (2001) 25/4 (463-471).
 Refs: 22
 ISSN: 1046-2023 CODEN: MTHDE

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
 029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Detection of single-nucleotide polymorphisms (SNPs) in high-throughput studies promises to be an expanding field of molecular medicine in the near future. Highly specific, simple, and accessible methods are needed to meet the rigorous requirements of single-nucleotide detection needed in pharmacogenomic studies, linkage analysis, and the detection of pathogens. Molecular beacons present such a solution for the high-throughput screening of SNPs in homogenous assays using the polymerase chain reaction (PCR). Molecular beacons are probes that fluoresce on hybridization to their perfectly complementary targets. In recent years they have emerged as a leading genetic analysis tool in a wide range of contexts from quantification of RNA transcripts, to probes on microarrays, to single-nucleotide polymorphism detection. The majority of these methods use PCR to obtain sufficient amounts of sample to analyze. The use of molecular beacons with other amplification schemes has been reliably demonstrated, though PCR remains the method of choice. Here we discuss and present how to design and use molecular beacons to achieve reliable SNP genotyping and allele discrimination in real-time PCR. In addition, we provide a new means of analyzing data outputs from such real-time PCR assays that compensates for differences between sample condition, assay conditions, variations in fluorescent signal, and amplification efficiency. The mechanisms by which molecular beacons are able to have extraordinary specificity are also presented. .COPYRGHT. 2001 Elsevier Science (USA).

L1 ANSWER 5 OF 16 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:259874 BIOSIS

DOCUMENT NUMBER: PREV200400258737

TITLE: Improving the accuracy of genetic diagnosis from single cells through analysis of real-time PCR signal kinetics.

AUTHOR(S): Pierce, Kenneth E. [Reprint Author]; Rice, John E. [Reprint Author]; Sanchez, Aquiles [Reprint Author]; Wangh, Lawrence J. [Reprint Author]

CORPORATE SOURCE: Brandeis Univ, Waltham, MA, USA

SOURCE: Fertility and Sterility, (September 2003) Vol. 80, No. Suppl. 3, pp. S9. print.
 Meeting Info.: 59th Annual Meeting of the American Society for Reproductive Medicine. San Antonio, Texas, USA. October 11-15, 2003. American Society for Reproductive Medicine.
 ISSN: 0015-0282 (ISSN print).

DOCUMENT TYPE: Conference; (Meeting)
 Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 19 May 2004
 Last Updated on STN: 19 May 2004

L1 ANSWER 6 OF 16 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

23 FILE VETU
 30 FILE WATER
 1 FILE WELDASEARCH
 1062 FILE WPIDS
 3 FILE WPIFV
 1062 FILE WPINDEX
 3 FILE WSCA
 L5 QUE (INTRAOPERATIVE OR OPERAT? OR SURGER? OR BIOPS?) AND (PCR
 OR POLYMERASE (W) CHAIN)

 D RANK

FILE 'EMBASE, MEDLINE, SCISEARCH, BIOSIS, BIOTECHNO, PASCAL, CAPLUS,
 CANCERLIT, ESBIOBASE, JICST-EPLUS, TOXCENTER, USPAT2, LIFESCI, PROMT,
 PATDPAFULL, IFIPAT, NLDB, BIOTECHDS, CABA, WPIDS, DRUGU, FRFULL, BIOENG,
 GENBANK, ADISCTI, DISSABS, COMPENDEX, ...' ENTERED AT 11:49:25 ON 02 FEB
 2005

L6 26928 SEA (INTRAOPERATIVE OR OPERAT? OR SURGER? OR BIOPS?) (14A)
 (PCR OR POLYMERASE (W) CHAIN)
 L7 758 SEA L6 AND (ULTRAFast OR RAPID OR FAST OR QUICK) (4A) (PCR OR
 POLYMERASE (W) CHAIN)
 L8 211 SEA L6 AND INTRAOPERATIVE
 L9 73 DUP REM L8 (138 DUPLICATES REMOVED)
 D 1-73 TI
 D 71 BIB AB
 D 69 BIB AB
 L10 381 SEA L6 AND (DURING (3A) SURGERY)
 L11 89 DUP REM L10 (292 DUPLICATES REMOVED)
 D 1-89 TI
 D 38 53 BIB AB
 L12 46 SEA L7 AND (INTRAOPERATIVE OR DURING (3A) SURGERY)
 L13 14 DUP REM L12 (32 DUPLICATES REMOVED)
 D 1-14 TI
 D 10-14 BIB AB

FILE HOME

FILE MEDLINE

FILE LAST UPDATED: 29 JAN 2005 (20050129/UP). FILE COVERS 1950 TO DATE.

On December 19, 2004, the 2005 MeSH terms were loaded.

Warning: The search L-number/HUMAN limit is missing from records indexed
 with the new 2005 MeSH (records added since December 19, 2004). Until
 this is corrected, include HUMANS/CT and 20041219-20051231/ED in
 searches to limit results to humans for this time period.

OLDMEDLINE now back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2005 vocabulary. See <http://www.nlm.nih.gov/mesh/> and
http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

This file contains CAS Registry Numbers for easy and accurate
 substance identification.

FILE BIOSIS

FILE COVERS 1969 TO DATE.

CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
 FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 26 January 2005 (20050126/ED)

FILE RELOADED: 19 October 2003.

FILE CAPLUS

Copyright of the articles to which records in this database refer is held by the publishers listed in the PUBLISHER (PB) field (available for records published or updated in Chemical Abstracts after December 26, 1996), unless otherwise indicated in the original publications. The CA Lexicon is the copyrighted intellectual property of the American Chemical Society and is provided to assist you in searching databases on STN. Any dissemination, distribution, copying, or storing of this information, without the prior written consent of CAS, is strictly prohibited.

FILE COVERS 1907 - 2 Feb 2005 VOL 142 ISS 6
FILE LAST UPDATED: 1 Feb 2005 (20050201/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE AGRICOLA

FILE COVERS 1970 TO 10 Jan 2005 (20050110/ED)

Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted material. All rights reserved. (2005)

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE ESBIODASE
FILE LAST UPDATED: 1 FEB 2005 <20050201/UP>
FILE COVERS 1994 TO DATE.

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN
/CC, /ORGN, AND /ST <<<

FILE JICST-EPLUS
FILE COVERS 1985 TO 24 JAN 2005 (20050124/ED)

THE JICST-EPLUS FILE HAS BEEN RELOADED TO REFLECT THE 1999 CONTROLLED TERM (/CT) THESAURUS RELOAD.

FILE AQUASCI
FILE COVERS 1978 TO 19 Jan 2005 (20050119/ED)

FILE STNGUIDE
FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Jan 28, 2005 (20050128/UP).

FILE STNINDEX

FILE EMBASE
FILE COVERS 1974 TO 27 Jan 2005 (20050127/ED)

EMBASE has been reloaded. Enter HELP RLOAD for details.

This file contains CAS Registry Numbers for easy and accurate

substance identification.

FILE SCISEARCH

FILE COVERS 1974 TO 27 Jan 2005 (20050127/ED)

FILE BIOTECHNO

FILE LAST UPDATED: 7 JAN 2004 <20040107/UP>

FILE COVERS 1980 TO 2003.

>>> BIOTECHNO IS NO LONGER BEING UPDATED AS OF 2004 <<<

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN
/CT AND BASIC INDEX <<<

FILE PASCAL

FILE LAST UPDATED: 31 JAN 2005 <20050131/UP>

FILE COVERS 1977 TO DATE.

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION IS AVAILABLE
IN THE BASIC INDEX (/BI) FIELD <<<

FILE CANCERLIT

FILE COVERS 1963 TO 15 Nov 2002 (20021115/ED)

On July 28, 2002, CANCERLIT was reloaded. See HELP RLOAD for details.

CANCERLIT thesauri in the /CN, /CT, and /MN fields incorporate the
MeSH 2002 vocabulary. Enter HELP THESAURUS for details.

This file contains CAS Registry Numbers for easy and accurate substance
identification.

FILE TOXCENTER

FILE COVERS 1907 TO 1 Feb 2005 (20050201/ED)

This file contains CAS Registry Numbers for easy and accurate substance
identification.

TOXCENTER has been enhanced with new files segments and search fields.
See HELP CONTENT for more information.

TOXCENTER thesauri in the /CN, /CT, and /MN fields incorporate the
MeSH 2005 vocabulary. See <http://www.nlm.nih.gov/mesh/> and
http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
description of changes.

FILE USPAT2

FILE COVERS 2001 TO PUBLICATION DATE: 1 Feb 2005 (20050201/PD)

FILE LAST UPDATED: 1 Feb 2005 (20050201/ED)

HIGHEST GRANTED PATENT NUMBER: US2004230467

HIGHEST APPLICATION PUBLICATION NUMBER: US2005021284

CA INDEXING IS CURRENT THROUGH 1 Feb 2005 (20050201/UPCA)

ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 1 Feb 2005 (20050201/PD)

REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2004

USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2004

USPAT2 is a companion file to USPATFULL. USPAT2 contains full text
of the latest US publications, starting in 2001, for the inventions
covered in USPATFULL. USPATFULL contains full text of the original
published US patents from 1971 to date and the original applications
from 2001. In addition, a USPATFULL record for an invention contains

a complete list of publications that may be searched in standard search fields, e.g., /PN, /PK, etc.

USPATFULL and USPAT2 can be accessed and searched together through the new cluster USPATALL. Type FILE USPATALL to enter this cluster.

Use USPATALL when searching terms such as patent assignees, classifications, or claims, that may potentially change from the earliest to the latest publication.

FILE LIFESCI
FILE COVERS 1978 TO 14 Jan 2005 (20050114/ED)

FILE PROMT
FILE COVERS 1978 TO 2 FEB 2005 (20050202/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE PATDPAFULL
FILE LAST UPDATED: 27 JAN 2005 <20050127/UP>
MOST RECENT UPDATE WEEK: 200504 <200504/EW>
FILE COVERS 1987 TO DATE

<<< PATDPAFULL HAS BEEN UPDATED.
HOWEVER, SOME BIBLIOGRAPHIC FIELDS ARE STILL MISSING.
---> SEE 'HELP CHANGE'.
THESE FIELDS WILL BE LOADED SOON. >>>

<<< PATDPAFULL WURDE FORTGESCHRIEBEN.
ALLERDINGS FEHLEN NOCH EINIGE BIBLIOGRAFISCHE FELDER.
---> SIEHE 'HELP CHANGE'.
DIESE WERDEN IN KUERZE NACHGELADEN. >>>

FILE IFIPAT
FILE COVERS 1950 TO PATENT PUBLICATION DATE: 1 Feb 2005 (20050201/PD)
FILE LAST UPDATED: 1 Feb 2005 (20050201/ED)
HIGHEST GRANTED PATENT NUMBER: US2005004268
HIGHEST APPLICATION PUBLICATION NUMBER: US2005022281
UNITERM INDEXING IS AVAILABLE IN THE IFIUDB FILE
UNITERM INDEXING LAST UPDATED: 31 Jan 2005 (20050131/UP)
INDEXING CURRENT THROUGH PAT PUB DATE: 27 Nov 2003 (20031127/PD)

INCL, INCLM, INCLS fields added. Please refer to ONLINE News for details.

FILE NLDB
FILE COVERS 1988 TO 2 FEB 2005 (20050202/ED)

FILE BIOTECHDS
FILE LAST UPDATED: 1 FEB 2005 <20050201/UP>

>>> USE OF THIS FILE IS LIMITED TO BIOTECH SUBSCRIBERS <<<

>>> NEW CLASSIFICATION SYSTEM FROM 2002 ONWARDS - SEE HELP CLA <<<

>>> NEW DISPLAY FIELDS LS AND LS2 (LEGAL STATUS DATA FROM
THE INPADOC DATABASE) AVAILABLE - SEE NEWS <<<

FILE CABA
FILE COVERS 1973 TO 7 Jan 2005 (20050107/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

The CABA file was reloaded 7 December 2003. Enter HELP RLOAD for details.

FILE WPIDS

FILE LAST UPDATED: 28 JAN 2005 <20050128/UP>
MOST RECENT DERWENT UPDATE: 200507 <200507/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
PLEASE VISIT:
http://www.stn-international.de/training_center/patents/stn_guide.pdf <<<

>>> FOR DETAILS OF THE PATENTS COVERED IN CURRENT UPDATES, SEE
<http://thomsonderwent.com/coverage/latestupdates/> <<<

>>> FOR INFORMATION ON ALL DERWENT WORLD PATENTS INDEX USER
GUIDES, PLEASE VISIT:
<http://thomsonderwent.com/support/userguides/> <<<

>>> NEW! FAST-ALERTING ACCESS TO NEWLY-PUBLISHED PATENT
DOCUMENTATION NOW AVAILABLE IN DERWENT WORLD PATENTS INDEX
FIRST VIEW - FILE WPIFV.
FOR FURTHER DETAILS: <http://www.thomsonderwent.com/dwpifv> <<<

>>> NEW DISPLAY FORMAT HITSTR ADDED ALLOWING DISPLAY OF
HIT STRUCTURES WITHIN THE BIBLIOGRAPHIC DOCUMENT <<<

>>> SMILES and ISOSMILES strings are no longer available as
Derwent Chemistry Resource display fields <<<

>>> THE CPI AND EPI MANUAL CODES HAVE BEEN REVISED FROM UPDATE 200501.
PLEASE CHECK:
<http://thomsonderwent.com/support/dwpioref/reftools/classification/code-rev>
FOR DETAILS. <<<

FILE DRUGU

FILE LAST UPDATED: 2 FEB 2005 <20050202/UP>
>>> DERWENT DRUG FILE (SUBSCRIBER) <<<

>>> FILE COVERS 1983 TO DATE <<<
>>> THESAURUS AVAILABLE IN /CT <<<

Sensitivity of **two-stage PCR**

amplification for detection of mycobacterium tuberculosis in paraffin-embedded tissues.

AUTHOR: Durmaz R.; Aydin A.; Durmaz B.; Aydin N.E.; Akbasak B.S.; Gunal S.
CORPORATE SOURCE: R. Durmaz, Department of Clinical Microbiology, Faculty of Medicine, Inonu University, Malatya, Turkey
SOURCE: Journal of Microbiological Methods, (1997) 29/2 (69-75).
Refs: 29
ISSN: 0167-7012 CODEN: JMIMDQ
PUBLISHER IDENT.: S 0167-7012(97)00022-5
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In order to improve the sensitivity of polymerase chain reaction (PCR) for the detection of mycobacterial DNA in paraffin-embedded tissues, a new approach with two sets of specific primers in **two-stage PCR** was employed in specimens obtained from tuberculosis patients and controls. Thirty-nine paraffin blocks selected from patients who had been diagnosed as having tuberculosis by radiological evaluations, histopathological findings, and clinical symptoms and signs including response to antituberculous treatment were examined. The control group consisted of 10 specimens from individuals that were proved to be negative for tuberculosis. After deparaffinization, lysis, phenol-chloroform extraction, and ethanol precipitation, the isolated DNA was amplified by PCR. Initially, all specimens were examined by the one-stage PCR using specific primers for 123-base pair (bp) fragment in IS6110 of mycobacterial DNA which yielded positive results only in 3 out of 39 (7.7%). In the **two-stage PCR** technique, 245 bp fragment of mycobacterial DNA was **amplified** at the first-step, then the PCR products were reamplified using the second specific **primer pairs** for 123-bp fragment. The true positivity of the **two-stage PCR** was 84.6% (33/39). The results indicate that **two-stage PCR** is more sensitive than one-stage (84.6% vs. 7.7%). All control specimens were negative by both PCR amplification methods, indicating that specificity of both methods was high. When the **two-stage amplification** was used, PCR positivity in the specimens obtained from different tissues was as follows: peritoneal and omental biopsies, 4/4; bone biopsies, 3/3; lymph node biopsies, 12/14; genito-urinary biopsies, 7/9; skin biopsies, 4/6; and one from each lung, breast, and pleural biopsies. PCR showed a good correlation with the granulomatous tissue reaction resulting in a 83.8% (31/37) positivity. The results indicate that the **two stage PCR amplification** can be used for detection of M. tuberculosis in paraffin-embedded tissues and is a useful technique in confirming tuberculosis in patients with clinically suspected disease who have acid-fast stain-negative.

Homogeneous multiplex genotyping of hemochromatosis

AUTHOR(S): mutations with fluorescent hybridization probes
Bernard, Philip S.; Ajioka, Richard S.; Kushner, James
P.; Wittwer, Carl T.
CORPORATE SOURCE: Departments of Pathology, University of Utah Medical
School, Salt Lake City, UT, 84132, USA
SOURCE: American Journal of Pathology (1998), 153(4),
1055-1061
CODEN: AJPAA4; ISSN: 0002-9440
PUBLISHER: American Society for Investigative Pathology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Multiplex polymerase chain reaction amplification and genotyping by
fluorescent probe melting temperature (T_m) was used to simultaneously
detect multiple variants in the hereditary hemochromatosis gene.
Homogenous real-time anal. by fluorescent melting curves has previously
been used to genotype single base mismatches; however, the current method
introduces a new probe design for fluorescence resonance energy transfer
and demonstrates allele multiplexing by T_m for the first time.
The new probe design uses a 3'-fluorescein-labeled probe and a
5'-Cy5-labeled probe that are in fluorescence energy transfer when
hybridized to the same strand internal to an unlabeled **primer**
set. Two hundred and fifty samples were genotyped for the C282Y
and H63D hemochromatosis causing mutations by fluorescent melting curves.
Multiplexing was performed by including two **primer sets**
and two probe sets in a single tube. In clin. defined groups of 117
patients and 56 controls, the C282Y mutation was found in 87% (204/234) of
patient chromosomes, and the relative penetrance of the H63D mutation was
2.4% of the homozygous C282Y mutation. Results were confirmed by
restriction enzyme digestion and agarose gel electrophoresis. In addition,
the probe covering the H63D mutation unexpectedly identified the A193T
polymorphism in some samples. This method is amenable to multiplexing and
has promise for scanning unknown mutations.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=>

ACCESSION NUMBER: 2002-16045 BIOTECHDS

TITLE: Parallel sequencing of DNA, useful e.g. for detecting point mutations, by nested **polymerase chain reaction**, using outer primers in solution and immobilized internal primers;
DNA sequencing, **polymerase chain reaction** and DNA array for diagnosis and genomics

AUTHOR: ZELTZ P; SCHNEIDER S

PATENT ASSIGNEE: BIOCHIP TECHNOLOGIES GMBH

PATENT INFO: EP 1186669 13 Mar 2002

APPLICATION INFO: EP 2000-119182 5 Sep 2000

PRIORITY INFO: EP 2000-119182 5 Sep 2000

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 2002-407210 [44]

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AB DERWENT ABSTRACT:

NOVELTY - Specific determination of DNA sequences (I), comprising parallel amplification in a combined liquid/solid phase microarray system, using nested **polymerase chain reaction** (PCR), with x **primer sets** (x = number of (I) being determined), each of at least three primers, is new.

DETAILED DESCRIPTION - Specific determination of DNA sequences (I), comprising parallel amplification in a combined liquid/solid phase microarray system, using nested **polymerase chain reaction** (PCR), with x **primer sets** (x = number of (I) being determined), each of at least three primers, is new. Each **primer set** comprises: (a) two outer primers (P1, P2) that hybridize upstream and downstream of the target DNA (A) being amplified; and (b) an internal primer (P3) that hybridizes to (A) and can form an extension product (EP). The outer primers are present in the liquid phase, at an excess relative to P3, and P3 are irreversibly bound to a solid phase, forming a microarray of x spaced apart and defined positions. Determination is based on detecting an EP from P3 at defined array positions. INDEPENDENT CLAIMS are also included for the following: (1) determining point mutations by the novel method; (2) determining the sequence of (unknown) partial sequences of DNA by the new method; and (3) solid phase DNA array of P3.

BIOTECHNOLOGY - Preferred Process: In (1), all x variants to be detected are amplified using P3x primer families in which each member has, at its 3'-end, a different nucleotide (nt). The annealing temperature is chosen so that an EP will be formed only at the position on the array where the primer is exactly complementary to the position being analyzed. In (2), the method is similar but each member of the P3x primer family differs from all others by 1 to n nt at the 3'-end (n = length of the sequence being determined), and annealing conditions are as in (1). When the partial sequence being determined is unknown, all n nt are identified and the set of P3 comprises all possible permutations. Many P3 will hybridize to different regions and generate EP. EP are determined, correlated with array positions and their sequences combined (exploiting overlap regions) to assemble a complete sequence. In all cases, the polymerase used lacks 3'-5'-exonuclease activity. The outer primers are used in 10 to the power 2-10 to the power 12, especially 10 to the power 4, times excess over P3 and have a different **melting temperature** from P3. Preferred Array: The solid support has a surface of metal (e.g. aluminum or gold); metal/semimetal oxide; glass or polymer. Where the surface is metal/semimetal oxide or glass, primers are attached through a bifunctional silane that has 1-3 hydrolyzable groups attached to silicon, e.g. halo, 1-4C alkoxy or acyloxy or amino. The silane may include a second functional group that can undergo e.g. a nucleophilic substitution or addition reaction. The array may include some or all of the other reagents required for the process, e.g.

polymerase, buffer etc., and the external primers are reversible dehydrated on to the surface. Particularly it is formulated as a 'laboratory on a chip', with additional systems, for heating. cooling etc. integrated into the array.

USE - The method is used to determine point mutations, to sequence (unknown) regions of DNA, e.g. in genomics or proteomics analysis, and for diagnostic determination of analytes.

ADVANTAGE - The method is an economical, simple and error-free way for amplifying an array of DNA sequences without compromising sensitivity or specificity. Unlike known nested PCR in the liquid phase, dilution and re-establishment of a reaction mixture after the first reaction are not required, i.e. the total analysis needs only one working operation. The use of high annealing temperatures minimizes non-specific binding, making it possible to measure increases in mass at specific locations by physical methods, e.g. from changes in refractive index.

EXAMPLE - No relevant examples are given. (21 pages)